

WHAT IS CLAIMED IS:

1. A composition for use in cloning or subcloning one or more desired nucleic acid molecules by recombinational cloning, comprising an effective amount of at least one ribosomal protein and an effective amount of at least one recombination protein.

2. The composition of claim 1, wherein said ribosomal protein is a prokaryotic ribosomal protein.

3. The composition of claim 1, wherein said ribosomal protein is an *Escherichia coli* ribosomal protein.

4. The composition of claim 1, wherein said ribosomal protein is a basic ribosomal protein.

5. The composition of claim 1, wherein said ribosomal protein has a molecular weight of less than about 14 kilodaltons.

6. The composition of claim 3, wherein said *E. coli* ribosomal protein is selected from the group of *E. coli* ribosomal proteins consisting of S10, S14, S15, S16, S17, S18, S19, S20, S21, L21, L23, L24, L25, L27, L28, L29, L30, L31, L32, L33 and L34.

7. The composition of claim 3, wherein said *E. coli* ribosomal protein is S20.

8. The composition of claim 3, wherein said *E. coli* ribosomal protein is L27.

9. The composition of claim 3, wherein said *E. coli* ribosomal protein is S15.

10. The composition of claim 1, wherein said recombination protein is a prokaryotic recombination protein.

11. The composition of claim 1, wherein said recombination protein is selected from the group consisting of Int, Cre, FLP, Xis, IHF and HU, and combinations thereof.

12. The composition of claim 1, wherein said recombination protein is Int.

13. The composition of claim 1, further comprising one or more nucleic acid molecules selected from the group consisting of one or more Insert Donor molecules, one or more Vector Donor molecules, one or more Cointegrate molecules, one or more Product molecules and one or more Byproduct molecules.

14. A method for cloning or subcloning one or more desired nucleic acid molecules comprising

- (a) forming a combination by combining *in vitro* or *in vivo*
- (i) one or more Insert Donor molecules comprising one or more desired nucleic acid segments flanked by at least two recombination sites, wherein said recombination sites do not substantially recombine with each other;
 - (ii) one or more Vector Donor molecules comprising at least two recombination sites, wherein said recombination sites do not substantially recombine with each other;
 - (iii) an effective amount of at least one recombination protein; and
 - (iv) an effective amount of at least one ribosomal protein; and

- (b) incubating said combination under conditions sufficient to transfer one or more of said desired segments into one or more of said Vector Donor molecules, thereby producing one or more desired Product nucleic acid molecules.

5
15. The method of claim 14, further comprising:

- (c) forming a combination by combining *in vitro* or *in vivo*

(i) one or more of said Product molecules comprising said desired segments flanked by two or more recombination sites, wherein said recombination sites do not substantially recombine with each other;

(ii) one or more different Vector Donor molecules comprising two or more recombination sites, wherein said recombination sites do not substantially recombine with each other;

(iii) an effective amount of at least one recombination protein; and

(iv) an effective amount of at least one ribosomal protein; and

- (d) incubating said combination under conditions sufficient to transfer one or more of said desired segments into one or more different Vector Donor molecules, thereby producing one or more different Product molecules.

10
15
20
25 16. The method of claim 14, wherein said ribosomal protein is a prokaryotic ribosomal protein.

17. The method of claim 15, wherein said ribosomal protein is a prokaryotic ribosomal protein.

30 18. The method of claim 14, further comprising incubating said different Product molecules with one or more different Vector Donor molecules

A3
cnc'd

Sub
B2

under conditions sufficient to transfer one or more of said desired segments into said different Vector Donor molecules.

- 5 Sub
act
- 10
- 15
- 20
19. A method for cloning or subcloning desired nucleic acid molecules comprising
- a) forming a combination by combining *in vitro* or *in vivo*
 - i) one or more Insert Donor molecules comprising one or more nucleic acid segments flanked by two or more recombination sites, wherein said recombination sites do not substantially recombine with each other;
 - ii) two or more different Vector Donor molecules comprising two or more recombination sites, wherein said recombination sites do not substantially recombine with each other;
 - iii) an effective amount of at least one recombination protein; and
 - iv) an effective amount of at least one ribosomal protein; and
 - b) incubating said combination under conditions sufficient to transfer one or more of said desired segments into said different Vector Donor molecules, thereby producing two or more different Product molecules.

25

20. The method of claim 19, wherein said ribosomal protein is a prokaryotic ribosomal protein.

21. The method of claim 14, wherein said ribosomal protein is an *Escherichia coli* ribosomal protein.

30

22. The method of claim 14, wherein said ribosomal protein is a basic ribosomal protein.

23. The method of claim 14, wherein said ribosomal protein has a molecular weight of less than about 14 kilodaltons.

24. The method of claim 21, wherein said *E. coli* ribosomal protein is selected from the group of *E. coli* ribosomal proteins consisting of S10, S14, S15, S16, S17, S18, S19, S20, S21, L21, L23, L24, L25, L27, L28, L29, L30, L31, L32, L33 and L34.

25. The method of claim 21, wherein said ribosomal protein is S20.

26. The method of claim 21, wherein said ribosomal protein is L27.

27. The method of claim 21, wherein said ribosomal protein is S15.

28. The method of claim 19, wherein said recombination protein is a prokaryotic recombination protein.

29. The method of claim 14, wherein said recombination protein is selected from the group consisting of Int, Cre, FLP, Xis, IHF, and HU, and combinations thereof.

30. The method of claim 14, wherein said recombination protein is Int.

31. A method for recombinational cloning of one or more desired nucleic acid molecules comprising

- (a) forming a mixture by mixing one or more of said desired nucleic acid molecules with one or more vectors and with the composition of claim 1; and
- (b) incubating said mixture under conditions sufficient to transfer said one or more desired nucleic acid molecules into one or more of said vectors.

32. The method of claim 31, wherein said desired nucleic acid molecules are derived from genomic DNA.

Ab conc 10
5 33. The method of claim 31, wherein said desired nucleic acid molecules are derived from cDNA.

34. The method of claim 31, wherein said desired nucleic acid molecules are produced by chemical synthesis.

10 35. The method of claim 31, wherein said desired nucleic acid molecules are produced by amplification.

36. The method of claim 31, wherein said vector is a prokaryotic or eukaryotic vector.

Sub 8a7
37. The method of claim 36, wherein said eukaryotic vector propagates and/or replicates in yeast cells, plant cells, fish cells, eukaryotic cells, mammalian cells, and/or insect cells.

38. The method of claim 31, wherein said prokaryotic vector propagates and/or replicates in bacteria of the genera *Escherichia*, *Salmonella*, *Bacillus*, *Streptomyces* or *Pseudomonas*.

25 39. The method of claim 38, wherein said prokaryotic vector propagates and/or replicates in *E. coli*.

30 40. A method for enhancement of recombinational cloning, comprising contacting a nucleic acid molecule with one or more ribosomal proteins and with one or more recombination proteins.

41. The method of claim 40, wherein said ribosomal protein is a prokaryotic ribosomal protein.

42. The method of claim 40, wherein said ribosomal protein is an *Escherichia coli* ribosomal protein.

43. The method of claim 40, wherein said ribosomal protein is a basic ribosomal protein.

44. The method of claim 40, wherein said ribosomal protein has a molecular weight of less than about 14 kilodaltons.

45. The method of claim 42, wherein said *E. coli* ribosomal protein is selected from the group of *E. coli* ribosomal proteins consisting of S10, S14, S15, S16, S17, S18, S19, S20, S21, L21, L23, L24, L25, L27, L28, L29, L30, L31, L32, L33 and L34.

46. The method of claim 42, wherein said ribosomal protein is S20.

47. The method of claim 42, wherein said ribosomal protein is L27.

48. The method of claim 42, wherein said ribosomal protein is S15.

49. The method of claim 40, wherein said recombination protein is a prokaryotic recombination protein.

50. The method of claim 40, wherein said recombination protein is selected from the group consisting of Int, Cre, FLP, Xis, IHF, and HU, and combinations thereof.

51. The method of claim 40, wherein said recombination protein is Int.

Sub
98

52. A DNA molecule produced by the method of claim 31.

53. The DNA molecule of claim 52, wherein said DNA molecule is an isolated DNA molecule.

54. A host cell comprising the DNA molecule of claim 52.

55. A kit for use in recombinational cloning of a nucleic acid molecule, said kit comprising at least one ribosomal protein and at least one recombination protein.

56. The kit of claim 55, wherein said ribosomal protein is a prokaryotic ribosomal protein.

57. The kit of claim 55, wherein said ribosomal protein is an *Escherichia coli* ribosomal protein.

58. The kit of claim 57, wherein said *E. coli* ribosomal protein is selected from the group of *E. coli* ribosomal proteins consisting of S10, S14, S15, S16, S17, S18, S19, S20, S21, L21, L23, L24, L25, L27, L28, L29, L30, L31, L32, L33 and L34.

a

59. The kit of claim 57, wherein said ribosomal protein is S20.

60. The kit of claim 57, wherein said ribosomal protein is L27.

61. The kit of claim 57, wherein said ribosomal protein is S15.

62. The kit of claim 55, wherein said recombination protein is a prokaryotic recombination protein.

64. The kit of claim 55, wherein said recombination protein is Int.

5

add a9

add B5